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MICROSCOPY.¹

THE PROCESS OF STAINING SECTIONS SIMPLIFIED BY MIXING THE STAINING FLUIDS WITH TURPENTINE.—According to Dr. Kükenthal's experiments, a large number of coloring substances admit of being mixed with turpentine, and serial sections may be stained in a short time by such a combination. Methyl-green, methyl-blue, gentian-violet, safranin, Bismarck-brown, eosin, fuchsin, tropæolin, and malachite-green may be used in this way.

The dry coloring substance is dissolved in *absolute* alcohol, and the solution dropped into turpentine until the mixture has any intensity of color desired.

Meyer's² Carmine Solution.

Absolute alcohol.....	100 cc.
Pulverized carmine.....	3 gr.
Hydrochloric acid (neutralized with ammonia)...	25 drops

Can be united with a mixture of turpentine and absolute alcohol [in equal parts?], and in this form used for staining sections.

The method of using these stains is very simple. The sections are fastened to the slide by Schällibaum's collodion, then left in the oven of the water-bath until the clove oil has been completely driven off. The paraffine is next removed by washing in turpentine, and then the slide is immersed in the staining mixture. As soon as the desired depth of stain has been received, the sections may be washed in pure turpentine and mounted in balsam.

If the stain is too deep, or a sharp nuclear stain is desired, it is only necessary to leave the slide a short time in a mixture of turpentine and pure (free from any trace of acid) absolute alcohol, and the color will be reduced.

The coloring mixture may become cloudy, as the result of the evaporation of the alcohol; in such an event, the addition of a drop or two of alcohol generally suffices to clear the mixture.

This method enables one to use easily several stains in succession. Objects may also be colored, *in toto*, with the advantage that the process of staining can be followed and easily controlled.

Fixing and Preserving Histological Preparations.—Dr. N. Kultschitzky³ discusses the merits and defects of the principal reagents employed in "fixing" and preserving histological preparations,

¹ Edited by C. O. Whitman, Director of the Lake Laboratory, Milwaukee.

² The carmine is boiled in the alcohol, and then the acid added. The solution is then filtered, hot, and enough ammonia added to neutralize. After filtering again the solution is mixed with turpentine and absolute alcohol.

³ Zeitschr. f. wiss. Mikroskopie, iv., 3, p. 345, 1887.

points out the requirements to be fulfilled by such reagents, lays down the principles by which one should be guided in selecting them, and concludes by giving a method which has proved to be eminently satisfactory.

Rules.—(a) For fixing tissues it is important to use reagents that do not form tissue-like precipitates with protoplasm. This requirement is met by *chromic salts, sulphate of copper, sublimate* and some other salts. *Preparations in chromic salts, when transferred to alcohol, should be kept in absolute darkness* (H. Virchow), until the fixing reagent is removed so far as possible.

(b) All reagents which transform protoplasm into tissue-like forms, as, *e.g.*, chromic acid, should be avoided, or their application must be controlled.

(c) Fixing fluids should contain an organic acid, *e.g.*, acetic acid, which changes nuclein into an insoluble state. The acid must be used in a diluted form, as nuclein is dissolved in strong acids.

The time of action must be short, as the long-continued action of even a weak acid dissolves nuclein.

(d) It is desirable that the fixing fluid should contain alcohol in a small quantity.

Strong alcohol dehydrates and induces changes in the protoplasm.

Kultschitzky's Fluid.—Add, *ad libitum*, pulverized bichromate of potassium and sulphate of copper to alcohol (50 per cent.). Keep in absolute darkness twenty-four hours. A transparent greenish-yellow fluid is thus obtained, which is to be acidulated before use with acetic acid (5 to 6 drops to 100 cc.).

Method.—Place the object in the fixing fluid for from twelve to twenty-four hours, according to its size and hardness, and keep in the dark; then transfer to strong alcohol. After twelve to twenty-four hours the preparation is hard enough for cutting.

Conservation.—Kultschitzky thinks that for conservation only such fluids should be used as produce no further changes in protoplasm after it has once been fixed. As alcohol, Müller's Fluid and other fluids in common use do work changes in the tissues, Kultschitzky recommends keeping preparations in ether, xylol, or toluol.

Accessory Nuclei (Nebenkerne, Paranuclei).—Dr. Gustav Platner¹ has been for some years engaged with the problem of the origin and meaning of accessory nuclei in gland-cells. The results of his work have not yet been published, so far as I am aware; but some of his methods of study have been given in the *Zeitschrift für wissenschaftliche Mikroskopie*, Vol. IV., No. 3, p. 349. Flemming's *chrom-osmio-acetic acid* is the best hardening, or "fixing" medium. This mixture may sometimes be modified to advantage by diminishing the quantity of acetic acid and increasing that of osmic acid. When the accessory nucleus forms a compact mass, as in reptiles

and many anura, a mixture of picric acid and sublimate gives good preparations.

A New Staining Fluid.—Finding that hæmatoxylin varied considerably in its effects, according to the age of the solution, or the method of hardening employed, Dr. Platner looked for another staining substance that would better meet his needs. The search led to the introduction of a new stain, for which Platner suggests the name "*nucleus-black*." This coloring substance is imported from Russia, and was obtained from the chemical laboratory of Dr. Grübler, Dufourstrasse, Leipzig.

A weak solution of nucleus-black stains only nuclei, nucleoli, and axis-cylinder, leaving the cytoplasm, connective tissue, and medullary sheath uncolored. Used at its full strength it stains other tissues, but with less intensity.

An over-stain is easily reduced by dilute ammonia (five or six drops to a watch-glass full of water), or, preferably, by *carbonate of lithium*, diluted *ad libitum*. A pure and intense nuclear stain may be thus obtained. Treated in this way, the accessory nuclei are stained in varying degrees of intensity, according to the stage of their development.¹

It is a remarkable fact that these accessory nuclei, soon after their formation, become non-receptive to *safranin* or *Victoria blue 4 R*, while remaining stainable with *nucleus-black*. It would seem, as Platner remarks, that chromatin is composed of two substances, one of which is affected only by certain nuclear stains, while the other is receptive to a large number of stains, and especially so to nucleus-black and hæmatoxylin.

Sections from preparations in Flemming's fluid may be left twenty-four hours in a dilute solution of nucleus-black. The time of exposure to the decoloring fluid will vary according to the intensity of the stain received and the end to be reached. The stain is permanent and well adapted to photographing.

The Eggs of Ascaris megalocephala.—Platner recommends heating to 50°C., for twenty to forty seconds, then hardening in ascending grades of alcohol. This method has the great advantage of killing instantly without injurious effects, and leaving the nuclear figures in a better state of preservation than can be reached by any other method hitherto used. The egg-sacks are placed in a test-tube plunged in a dish of hot water. This method will undoubtedly be useful in other cases.

¹ Accessory nuclei arise from the chromatin of the nucleus, by a process of budding, and their development may be induced by starving the animal. On the sixth or seventh day, in the case of the salamander, the formation begins, and by the end of eight or nine days one or more accessory nuclei may be found in almost every gland-cell. As soon as the cells begin again to secrete, the accessory nuclei become pale and then disappear.

Bobretzky, Hertwig, Reichenbach, and others who have employed the method of heating, have subjected the eggs to a heat of 80°C ., or more, and for a considerable length of time. Platner is unquestionably right in attributing previous failures in the use of this method to the unnecessarily high temperature employed. Max Schultze has shown that protoplasm is killed and stiffened at 50°C ., and the use of a nearly boiling heat is therefore quite as unnecessary as it is harmful.

PARAFFINE PREPARED FOR RIBBON-CUTTING.—Dr. Spee² finds that paraffine prepared in the following manner is best adapted to ribbon-cutting:—

Take paraffine, which melts at about 50°C ., and melt it over a spirit lamp. Keep hot for from one to six hours, until it assumes a brownish yellow color, like that of yellow wax or honey. When cold the mass is perfectly homogeneous, and without air-bubbles. Sections, if not over $\frac{1}{100}$ mm. thick, stick firmly together in the form of a ribbon.

² *G. F. Spee*. Leichtes Verfahren zur Erhaltung linear geordneter, lückenloser Schnittserien mit Hülfe von Schnittbändern. Zeitschr. f. wiss. Mikroskopie, ii., 1, p. 7, 1885.

SCIENTIFIC NEWS.

—The well-known traveler and Siberian explorer, Nikolai Michalowitsch Prjewalsky, died November 1st, in Karakul.

—Mr. T. H. Potts, an ornithologist, who has done much for the exploration of the New Zealand Fauna, has recently died.

—Professor Joseph F. James, M.S., formerly of Miami University, Oxford, Ohio, should be addressed after September 10, 1888, Agricultural College, Prince George's County, Maryland.

—The Lowell Institute free courses of lectures to the teachers of Boston begin January 5th, with a course by Prof. W. O. Crosby, of the Boston Society of Natural History, upon the geology of Boston and its vicinity. The course consists of (1) a general study of the physical features of the Boston Basin, and of the geological changes now in progress; (2) a systematic study of the various minerals and rocks found in the Boston Basin, together with the more characteristic kinds of structure which they exhibit; (3) a